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Exploring mutasynthesis to increase structural diversity in the synthesis of highly oxygenated polyketide lactones†

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The enantioselective synthesis of (2*R*,3*R*,4*E*,8*E*)-3-hydroxy-2,4,8-trimethyldeca-4,8-dienolide (**5**) by ring-closing metathesis is described. This compound is an analogue of 3,4-dihydroxy-2,4,6,8-tetramethyldec-8-enolide (**4**) which is a rare 11-membered lactone produced by the fungus, *Botrytis cinerea*. Mutasynthetic studies with compound **5** using two mutants of *B. cinerea* led to the isolation of four new highly oxygenated 11-membered lactones (**11–14**) in which compound **5** has been stereoselectively epoxidized and hydroxylated at sites that were not easily accessible by classical synthetic chemistry.

Introduction

Botrytis cinerea is a grey powdery phytopathogenic mould that affects a large number of commercial crops.[1] Its major phytotoxic metabolites are a family of sesquiterpenes with the botryane carbon skeleton[2] and two groups of polyketides exemplified, on the one hand, by the botcinic and botcineric acids (**1**) and their cyclic derivatives, the botcinins (**2**),[3] and on the other hand, by botrylactone (**3**).[4] A related lactone, 3,4-dihydroxy-2,4,6,8-tetramethyldec-8-enolide (**4**), has been isolated[5] from a mutant strain of *B. cinerea*. This lactone possesses the same carbon chain as botrylactone with identical functional groups and stereochemistry in the C1–C4 fragments as both the botcinins and botrylactone. This structural homology and the presence of compound **4** in cultures of the strains of *B. cinerea* which also produce large amounts of the botcinins suggest that they have a common biosynthetic origin. Compound **4** may be a 'shunt' metabolite in this pathway (Fig. 1).

The sequencing of the *B. cinerea* genome led to the development of genetically modified strains lacking the genes which code for the enzymes that are involved in the biosynthesis of the secondary metabolites produced by this fungus.[6] Studies of the metabolites produced by these mutants permitted the identification of the genes involved in the production of the polyketides.[7] The genes responsible for the formation of the per-methylated tetraketide core of both botcinins (**2**) and botrylactones (**3**) are all included in the same cluster comprising genes *BcBOA1* to *BcBOA6*. The latter encodes the polyketide synthase (PKS).[6]

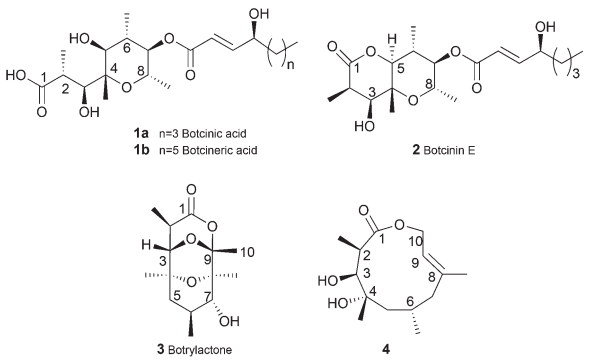

Fig. 1 Some polyketides produced by *B. cinerea*.

Naturally-occurring medium-sized lactones (8 to 11-membered lactones) are a relatively small number of metabolites which have nevertheless attracted considerable attention because of the range of biological activities which they possess.[8] In particular there are only a few examples of 11-membered lactones that are found in nature.[9] Apart from compound **4** and some complex pyrrolizidine and daphniphyllum alkaloids, there are only reports of two insect pheromones, ferrulactone I[10] and suspensolide,[11] and four fungal metabolites including the aspercyclides A–C.[12] The latter have anti-inflammatory activity and have potential to be used in the treatment of allergic disorders such as asthma.

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†Electronic supplementary information (ESI) available: Copies of the ^{1}H NMR and ^{13}C NMR spectra for all key intermediates and final products, 2D NMR of **11–14** as well as nOe spectra of (*E*)-**5** and **11–14**. See DOI: 10.1039/c4ob00717d

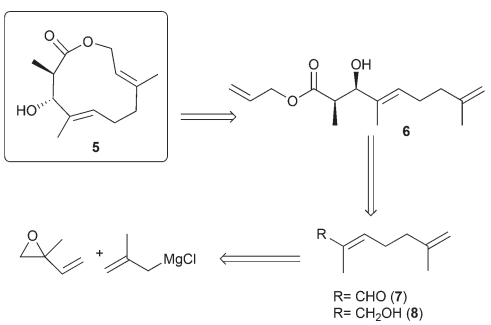
The relative thermodynamic instability of 11-membered lactones has made their synthesis and that of their derivatives difficult even using Mitsunobu lactonization[13] or an intramolecular Reformatsky reaction.[14] Recently, ring-closing metathesis (RCM) has been developed to provide an efficient method for the preparation of some 11-membered lactones from acyclic precursors.[15] However RCM reactions in the macrocyclic series tend to give mixtures of the (*E*) and (*Z*) isomers of cyclic olefins. A reliable general method for controlling the geometry of the new double bond is yet to be found.[16] Consequently there are few examples of synthetic analogues whose biological activity has been thoroughly evaluated. Although botrylactone was described by Welmar *et al.*[17] as a powerful antibiotic whilst the botcinins have been reported[18] to show antifungal activity against *Magnaporthe grisea*, the causal agent of rice blast, nothing is known of the biological activity of compound **4** or its close relatives because of the small amount of material that has been isolated.

Mutasynthesis is a very interesting strategy that combines chemical synthesis with biosynthetically-patterned biotransformations using genetically engineered microorganisms.[19] The availability of mutants of *B. cinerea* blocked in the early stages of the pathway but retaining some of the later stages leading to these 11-membered lactones has allowed us to explore the use of mutasynthesis to generate modified 11-membered lactones that are analogues of compound **4** in order to evaluate their biological activity.

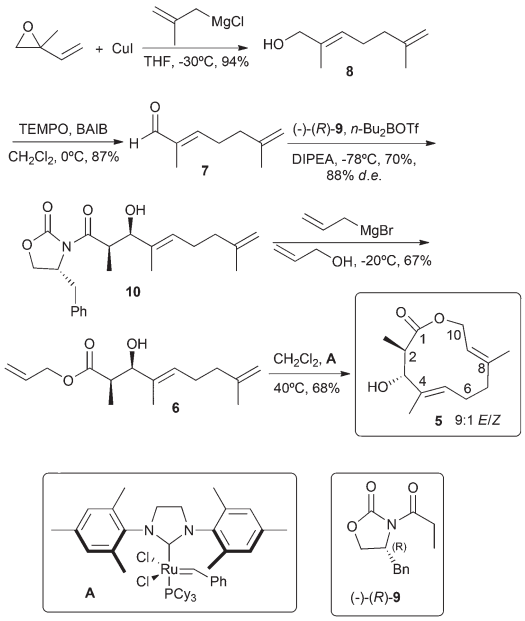
Results and discussion

We chose compound **5** as a simplified analogue of compound **4** although the stereochemistry of the alcohol at C-3 is different. However it could be easily synthesized in sufficient quantity for the mutasynthesis experiments. The retrosynthetic analysis of the lactone is shown in Scheme 1. An RCM cyclization of the ester **6** and a *syn* aldol reaction of the aldehyde **7** play key roles in the stereoselective construction of the 11-membered lactone ring.

The synthetic sequence leading to the lactone **5** is shown in Scheme 2. 2-Methyl-2-vinyloxirane was treated with commercially available 2-methylallyl magnesium chloride in the presence of CuI to produce the allylic alcohol **8** in a yield of 95%.[20]



Scheme 1 Retrosynthetic analysis of lactone **5**.



Scheme 2 Stereoselective synthesis of **5**.

Oxidation of **8** with TEMPO-BAIB (2,2,6,6-tetramethyl piperidinyloxy-bis(acetoxy)iodobenzene)[21] afforded the aldehyde **7**. This was then treated with the boron *Z*-enolate of the *N*-propionyl oxazolidinone (−)-(*R*)-**9**[22] to give the *syn*-aldol product **10** in 70% yield and 88% de.‡ Exocyclic cleavage of the oxazolidinone **10** with allyl alcohol and 4 eq. of allylmagnesium bromide at −20 °C generated the ester **6** with a yield of 67%. Finally an RCM reaction of **6** under high dilution conditions catalysed by the second-generation ruthenium complex **A**[23] in dry, degassed, refluxing dichloromethane produced the 11-membered lactone **5** with a good regioselectivity and yield (9 : 1 *E*/*Z* ratio, 68% yield).§‡ This is in agreement with molecular mechanics calculations (MM2) which predict that the (*E*)-alkene is the most stable regioisomer.[24]

The lactone **5** was then incubated with two mutant strains of *B. cinerea*, *bcbot2Δ* and *bcboa6Δbcbot2Δ* (*bcΔΔd1*). The first mutant, which was obtained by deactivating the botryane biosynthesis gene *BcBOT2* that encodes a sesquiterpene cyclase,[25] does not produce the botryanes but does produce a significant amount of botcinic acid (**1a**) and its relatives. The second mutant, *bcΔΔd1*,[6] is a double mutant which was obtained by deactivating the genes *BcBOT2* and *BcBOA6* and is consequently unable to produce the three characteristic families of metabolites: the botryanes, the botcinins and the botrylactone.[6] Incubation of compound **5** with both mutant strains using liquid surface-culture conditions gave four new 11-membered lactones **11**–**14** (Fig. 2). Their distribution and % yields are shown in Table 1. The strain *bcbot2Δ* gave a higher conversion of compound **5**. The metabolites **11**, **12** and **13** were

‡ Ratio valuated using ^{1}H-NMR.

§ It is noteworthy that with the first-generation ruthenium catalyst PhCH=$RuCl_2(PCy_3)_2$, a linear dimer was obtained after 16 h at reflux in CH_2Cl_2.

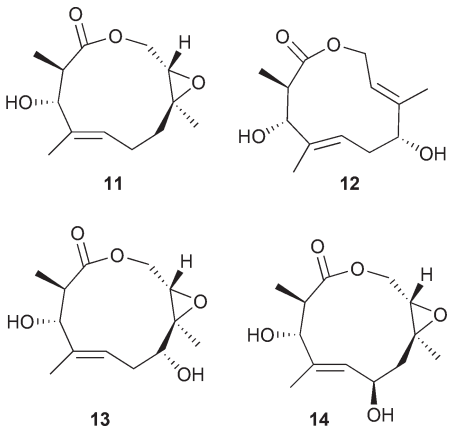


Fig. 2 Metabolites isolated in mutasynthesis.

Table 1 Distribution and yields of isolated metabolites

Strains	Metabolites (% yields)
bcbot2Δ	**5** (3), **11** (5), **12** (4), **13** (19), **14** (1)
bcΔΔd1	**5** (23), **11** (15), **12** (7), **13** (7)

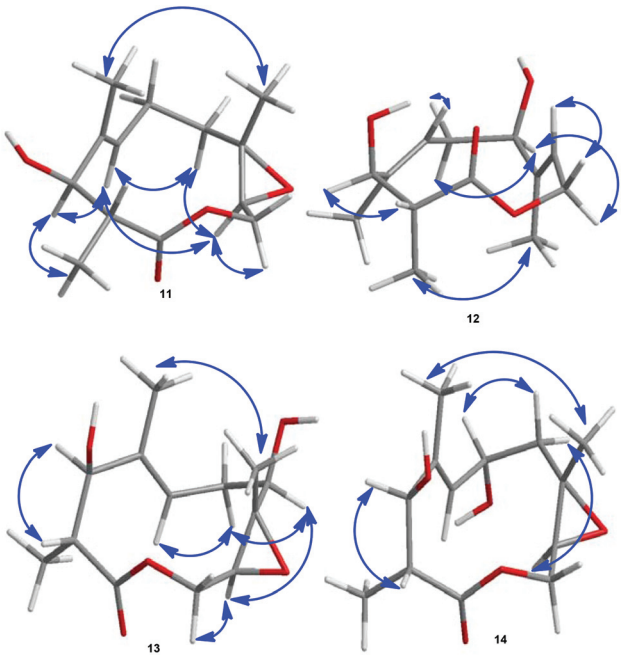


Fig. 3 MM2 minimized models with decisive nOe correlations for compounds **11–14**.

produced in both cases but there was no apparent incorporation of compound **5** into the botcinin/botrylactone pathway. The structures of the metabolites were established from their ^{1}H and ^{13}C NMR spectra using a combination of 1D and 2D NMR experiments (see ESI†).

Compound **11** was obtained as a colourless oil whose HRMS possessed a molecular ion at *m*/*z* 240.1353 corresponding to the molecular formula $C_{13}H_{20}O_4$. The ^{13}C NMR spectrum contained resonances at δ_C 81.6, 62.9, 59.8 and 58.6 ppm (CH, CH_2, C, and CH carbons respectively) and at δ_C 174.4 ppm (C carbon) consistent with the presence of four C–O and one lactone C═O carbon atoms in the molecule. The main difference between the ^{1}H NMR spectra of compounds **5** and **11** was the absence of the olefinic proton H-9 and the appearance of a double-doublet (*J* 10.0 and 4.2 Hz) at δ_H 3.01 ppm in compound **11**. The presence of an epoxide at this position was confirmed by an HSQC correlation of H-9 with the signal at δ_C 58.6 ppm (C-9) and HMBC correlations between H-9 and signals at δ_C 59.8 (C-8) and δ_C 62.9 ppm (C-10). The stereochemistry of the oxirane ring was established by nOe experiments which were rationalized on the basis of the lowest energy optimized conformer derived from MM2 calculations (Fig. 3).[24] In particular there were interactions between H-9 and δ_H 5.17 (H-5), δ_H 2.16–2.08 (H-6β), and δ_H 1.08 (H-7β) and between the signals at δ_H 1.75 (4-Me) and δ_H 1.31 (8-Me). There were also nOe effects involving the signals at δ_H 3.99 (H-3β) and δ_H 1.28 (2-Meβ). These were consistent with the epoxidation of compound **5** on the β-face of the ring.[26] Based on the known absolute configuration of compound **5** and the MM2 calculations, compound **11** has the absolute configuration (2*R*,3*R*,4*E*,8*S*,9*S*).

The HRMS (*m*/*z* 239.1282, M − H^+) of compound **12** was consistent with the molecular formula $C_{13}H_{20}O_4$. The ^{13}C NMR spectrum contained 13 signals. A comparison with compound **5** showed that the resonance for C-7 [δ_C 38.2 (t)] had changed to δ_C 77.2 (d) in compound **12**. There were COSY correlations between δ_H 4.18 (H-7) and the signals at δ_H 2.46 and 2.30 which were assigned to H-6α and H-6β respectively. There were nOe effects which were observed (Fig. 3) between the signals at δ_H 4.18 (H-7), 5.62 (H-9), 5.02 (H-5) and H-6β, together with δ_H 4.70 (H-10β) and H-9. These were consistent with the *R* configuration at C-7 and hence compound **12** was (2*R*,3*R*,4*E*,7*R*,8*E*)-3,7-dihydroxy-2,4,8-trimethyldeca-4,8-dienolide.

The HRMS data for compounds **13** and **14** showed that they both had a molecular formula, $C_{13}H_{20}O_5$. Their ^{13}C NMR spectra contained five C–O signals at δ_C 81.4 (CH, C-3), 76.9 (CH, C-7), 62.6 (C, C-8), 62.2 (CH_2, C-10) and 56.6 (CH, C-9) ppm for compound **13** and δ_C 80.9 (CH, C-3), 65.9 (CH, C-6), 62.8 (CH_2, C-10), 58.6 (CH, C-9) and 58.0 (C, C-8) ppm for **14**. These compounds possessed similar ^{1}H NMR spectra in which the olefinic C–H of compound **5** had been replaced by epoxy C–H signals at δ_H 3.09 (dd, *J* 10.0, 4.0 Hz) and δ_H 3.05 (dd, *J* 10.2, 4.2 Hz) respectively. However compound **13** possessed a new CH(OH) signal at δ_H 3.22 (dd, *J* 11.3, 5.2 Hz) (H-7) which showed COSY correlations with signals at δ_H 2.50 (H-6α) and δ_H 2.32 (H-6β). Compound **14** possessed a new CH(OH) signal at δ_H 4.64 (triplet *J* 11.2 of doublets *J* 4.8 Hz) (H-6) which showed COSY correlations with signals at δ_H, 2.48 (H-7β), δ_H 1.12 (H-7α) and 5.20 (H-5). The nOe effects, shown in Fig. 3, were in accord with the optimized structures obtained by MM2 calculations[24] and, bearing in mind the origin of the metabolites, led to the absolute stereochemistry for compounds **13** and **14** as 2*R*,3*R*,4*E*,7*R*,8*S*,9*S* and 2*R*,3*R*,4*E*,6*R*,8*S*,9*S* respectively.

These mutasynthetic transformations have produced derivatives that were hydroxylated at C-6 and C-7 and regioselectively epoxidized at C-8/C9. However there was no hydration of epoxidation of the C4/C5 double bond. Consequently additional synthetic work is necessary in order to develop an efficient route for the synthesis of closer analogues of compound **4**.

Compounds **11** and **13** did not show any significant anti-bacterial activity at a concentration of 200 ppm against *Escherichia coli*, *Bacillus subtilis* or *Streptococcus faecalis*.[27] Compounds **11** and **13** did not show any significant phytotoxic activity when tested *in vivo* on sterilized leaves of *Phaseolus vulgaris* at a concentration of 500 ppm.[28]

Conclusions

The regioselective ring-closing metathesis of the triene **6** has provided an efficient method for the asymmetric synthesis of (2*R*,3*R*,4*E*,8*E*)-3-hydroxy-2,4,8-trimethyldeca-4,8-dienolide (*E*)-**5** in 5 steps with 26% overall yield from simple starting materials. This compound is an advanced analogue of 3,4-dihydroxy-2,4,6,8-tetramethyldec-8-enolide (**4**) which is a scarce metabolite of *B. cinerea*. Compound **5** was biotransformed by two mutant strains of *B. cinerea*, *bcbot2Δ* and *bcΔΔd1*, into four new highly oxygenated 11-membered lactones. These mutasynthetic steps involved regioselective epoxidation of the C8/C9 double bond and hydroxylation at either the C-6 (minor) or C-7 (major) positions. This has proved to be a valuable and versatile approach to increasing the structural diversity of 11-membered lactones. Further experiments are in progress to develop a synthesis method of compound **4**.

Experimental

General procedures

Unless otherwise noted, materials and reagents were obtained from commercial suppliers and were used without further purification. Dichloromethane was freshly distilled from CaH_2 and tetrahydrofuran was dried over sodium and benzophenone and freshly distilled before use. Air- and moisture-sensitive reactions were performed under an argon atmosphere. Purification by semipreparative and analytical HPLC was performed with a Hitachi/Merck L-6270 apparatus equipped with a differential refractometer detector (RI-7490). A LiChrospher® Si 60 (5 μm) LiChroCart® (250 mm × 4 mm) column and a LiChrospher® Si 60 (10 μm) LiChroCart® (250 mm × 10 mm) column were used in isolation experiments. Silica gel (Merck) was used for column chromatography. TLC was performed on a Merck Kiesegel 60 F_{254}, 0.25 mm thick. Melting points were measured with a Reichert-Jung Kofler block and are uncorrected. Optical rotations were determined with a digital polarimeter. Infrared spectra were recorded on a FT-IR spectrophotometer and reported as wave number (cm^{-1}). ^1H and ^{13}C NMR measurements were recorded on Varian Unity 400 MHz, Agilent 500 MHz and Varian Inova 600 MHz spectrometers with $SiMe_4$ as the internal reference at room temperature. *J* values are given in Hz. Chemical shifts were referenced to $CDCl_3$ (δ_H 7.25, δ_C 77.0). NMR assignments were made using a combination of 1D and 2D techniques. Multiplicities are described using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quarter; quint = quintuplet; sext = sextuplet; m = multiplet, br = broad. High-Resolution Mass Spectroscopy (HRMS) was recorded with a double-focusing magnetic sector mass spectrometer in positive ion mode or with a QTOF mass spectrometer in positive ion electrospray mode at 20 V cone voltage. HRESIMS/MS experiments were performed with a QTOF mass spectrometer at 20 V cone voltage and 10 eV collision energy.

Microorganisms

B. cinerea mutants, *bcbot2Δ and bcΔΔd1*, were previously obtained by deactivating the genes encoding the sesquiterpene synthase BcBOT2,[25] the synthase BcBOT2 and the polyketide synthase BcBOA6,[6] respectively, and are maintained in the BIOGER strain collection INRA (Grignon, France). Conidial stock suspensions of these strains were maintained in glycerol (80%) at −40 °C.

Synthesis of the substrates

(*E*)-2,6-Dimethylhepta-2,6-dien-1-ol (8). 2-Methylallylmagnesium chloride (58.4 mL of a 0.5 M solution in THF, 29.2 mmol) at −30 °C was added to a stirred solution of 2-methyl-2-vinyloxirane (2 mL, 20.9 mmol) and CuI (199 mg, 1.04 mmol) in dry THF (22 mL) at −30 °C. The mixture was stirred at −30 °C and, after 3 h, was treated with a saturated ammonium chloride solution (60 mL) and then allowed to warm to room temperature. The aqueous layer was extracted three times with diethyl ether (90 mL). Combined extracts were washed with 1 N HCl (200 mL), saturated sodium bicarbonate (200 mL), water (200 mL), and brine (200 mL) and then dried over anhydrous sodium sulphate. Finally, the solvent was concentrated under reduced pressure and the crude was purified by silica gel column chromatography eluted with pentane–Et_2O (80 : 20) to yield compound **8** (2756 mg, 94%) as a colourless oil. Spectroscopic data of compound **8** were identical to those described in the literature.[20]

(*E*)-2,6-Dimethylhepta-2,6-dienal (7). TEMPO (890 mg, 5.6 mmol) and (diacetoxyiodo)benzene (BAIB, 18.3 g, 83.7 mmol) were added at 0 °C to a solution of (*E*)-2,6-dimethylhepta-2,6-dien-1-ol (**8**) (3.9 g, 27.9 mmol) in dry CH_2Cl_2 (62 mL). The mixture was stirred for 6 h and the solvent was concentrated under reduced pressure. The crude was purified by silica gel column chromatography eluted with pentane–Et_2O (96 : 4) to yield the aldehyde **7** (3339 mg, 87%) as a yellow oil. IR (film) ν_{max}/cm^{-1} 2925, 2852, 2769 (CHO), 1702 (CO), 1420, 1102, 1090; ^1H NMR (400 MHz, $CDCl_3$) δ_H 1.71 (6H, s, 2-Me, 6-Me), 2.20 (2H, t, *J* 7.2, 5-H), 2.49 (2H, dq, *J* 7.2, 0.6, 4-H), 4.70 (1H, br s, 7a-H), 4.77 (1H, br s, 7b-H), 6.47 (1H, tq, *J* 7.2, 1.0, 3-H), 9.38 (1H, s, 1-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 9.1 (q, 2-Me), 22.2 (q, 6-Me), 26.8 (t, C4), 36.0 (t, C5), 110.8 (t, C7), 139.3 (s, C2), 143.9 (s, C6), 153.9 (d, C3), 195.0

(s, C1); HRMS (CI^+): calcd for $C_9H_{14}O$ $[M]^+$ 138.1044, found 138.1028.

(4*R*,2″*R*,3″*R*,4″*E*)-4-Benzyl-3-[3-hydroxy-2,4,8-trimethylnona-4,8-dienoyl]-oxazolidin-2-one (10). *n*-Dibutylboron triflate (5.2 mL of a 1.0 M solution in CH_2Cl_2, 5.2 mmol) was added dropwise at 0 °C to a stirred solution of (−)-(4*R*)-4-benzyl-3-propionyloxazolidin-2-one ((−)-(*R*)-**9**) (1020 m, 4.36 mmol) in dry CH_2Cl_2 (6.3 mL) under argon atmosphere conditions. The mixture was stirred for 5 min and then *N*,*N*′-diisopropylethylamine was added dropwise (0.96 mL, 5.7 mmol). After complete addition, the mixture was stirred at 0 °C for 15 min. The yellow solution was re-cooled to −78 °C and a solution of (*E*)-2,6-dimethylhepta-2,6-dienal (**7**) (1220 mg, 8.71 mmol) was added dropwise in dry CH_2Cl_2 (2.0 mL). The mixture was stirred at −78 °C and was then allowed to warm to 0 °C and stirred for an additional hour. The reaction was quenched with a mixture of phosphate buffer (5 mL of a 1.0 M solution at pH 7) and MeOH (15 mL) at 0 °C and the mixture was stirred for 5 min. Finally, a solution of 2.4 : 1 MeOH–H_2O_2 (30%, 15 mL) was added slowly at 0 °C and stirred for an additional hour. The solvent was concentrated under reduced pressure and the residue was re-dissolved in diethyl ether. The aqueous layer was extracted three times with diethyl ether (35 mL). Combined extracts were washed with brine (200 mL), dried over anhydrous sodium sulphate, filtered, the solvent evaporated and the crude was purified by silica gel column chromatography eluted with petroleum ether–ethyl acetate (75 : 25) to yield compound **10** (1126 mg, 70%) as a white solid. m. p. 37 °C (from CH_2Cl_2); $[\alpha]_D^{20}$ −41.3° (*c* 0.65 in $CHCl_3$); IR (film) ν_{max}/cm^{-1} 3524 (OH), 3176, 2935, 1781 (CO), 1698 (CO), 1456, 1385, 1208, 1107, 886, 747; ^1H NMR (400 MHz, $CDCl_3$) δ_H 1.16 (3H, d, *J* 6.8, 2′-Me), 1.60 (3H, s, 4′-Me), 1.70 (3H, s, 8′-Me), 2.05 (2H, t, *J* 7.4, 7′-H), 2.18 (2H, q, *J* 7.4, 6′-H), 2.77 (1H, dd, *J* 13.4, 9.2, C*H*HPh), 2.88 (d, *J* 2.8, 3′-O*H*), 3.24 (1H, dd, *J* 13.4, 3.2, CH*H*Ph), 3.95 (1H, dq, *J* 6.8, 3.6, 2′-H), 4.18 (2H, m, 5a-H, 5b-H), 4.33 (1H, br s, 3′-H), 4.64–4.66 (2H, m, 4-H, 9′a-H), 4.69 (1H, br s, 9′b-H), 5.52 (1H, t, *J* 7.4, 5′-H), 7.17–7.33 (5H, m, *H*arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 10.4 (q, 2′-Me), 13.3 (q, 4′-Me), 22.3 (q, 8′-Me), 25.7 (t, C6′), 37.4 (t, C7′), 37.6 (t, *C*H$_2$Ph), 40.4 (d, C2′), 55.2 (d, C4), 66.0 (t, C5), 75.1 (d, C3′), 109.9 (t, C9′), 125.7 (d, C5′), 127.3 (*C*arom), 128.8 (2C, *C*arom), 129.3 (2C, *C*arom), 133.6 (s, C4′), 135.0 (s, *C*arom), 145.3 (s, C8′), 152.9 (s, C2), 176.9 (s, C1′); HRMS (CI^+): calcd for $C_{22}H_{29}NO_4$ $[M]^+$ 371.2097, found 371.2088.

(2*R*,3*R*,4*E*)-Allyl 3-hydroxy-2,4,8-trimethylnona-4,8-dienoate (6). Allylmagnesium bromide (4.9 mL of a 1.0 M solution in Et_2O, 4.9 mmol) was added at 0 °C to alcohol (15 mL) under argon conditions in a Schlenk flask. The allylic mixture was stirred for 10 min and re-cooled at −20 °C. Then, a solution of **10** (886 mg, 2.40 mmol) in allylic alcohol (3 mL) was slowly added. When TLC monitoring indicated the completion of the reaction (3 h), a saturated ammonium chloride solution (20 mL) was added and then allowed to warm to room temperature. The aqueous layer was extracted three times with diethyl ether (50 mL). Combined extracts were washed with brine (100 mL), dried over anhydrous sodium sulphate, filtered and the solvent was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography eluted with petroleum ether–Et_2O (90 : 10) to yield the ester **6** (405 mg, 67%) as a yellow oil. $[\alpha]_D^{20}$ +3.7° (*c* 0.42 in $CHCl_3$); IR (film) ν_{max}/cm^{-1} 3448 (OH), 2924, 1736 (CO), 1458, 1376, 1170, 1032, 888; ^1H NMR (400 MHz, $CDCl_3$) δ_H 1.15 (3H, d, *J* 7.1, 2-Me), 1.60 (3H, s, 4-Me), 1.71 (3H, s, 8-Me), 2.04 (2H, t, *J* 7.6, 7-H), 2.13–2.20 (2H, m, 6-H), 2.25 (d, *J* 4.4, 3-O*H*), 2.70 (1H, dq, *J* 7.1, 4.4, 2-H), 4.27 (1H, t, *J* 4.4, 3-H), 4.57 (2H, dt, *J* 5.6, 1.4, 1′-H), 4.66 (1H, br s, 9b-H), 4.70 (1H, br s, 9a-H), 5.23 (1H, ddd, *J* 10.4, 2.8, 1.4, 3′b-H), 5.31 (1H, ddd, *J* 17.4, 2.8, 1.4, 3′a-H), 5.47 (1H, t, *J* 7.0, 5-H), 5.90 (1H, ddt, *J* 17.4, 10.4, 5.6, 2′-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 11.3 (q, 2-Me), 12.7 (q, 4-Me), 22.5 (q, 8-Me), 25.8 (t, C6), 37.4 (t, C7), 43.0 (d, C2), 65.2 (t, C1′), 76.8 (d, C3), 110.0 (t, C9), 118.3 (t, C3′), 126.6 (d, C5), 132.0 (d, C2′), 134.0 (s, C4), 145.4 (s, C8), 175.1 (s, C1); HRMS (CI^+): calcd for $C_{15}H_{24}O_3$ $[M]^+$ 252.1725, found 252.1718.

RCM of 6. (1,3-Bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(phenylmethylene)(tricyclohexyl-phosphine) ruthenium (Grubbs ruthenium catalyst **A**, 121.3 mg, 0.14 mmol) was added to a refluxed and stirred solution of ester **6** (180.0 mg, 0.71 mmol) in degassed and dry CH_2Cl_2 (420 mL) under argon conditions. The reaction mixture was stirred until consumption of the starting material (20 h). The crude was filtered over a pad of silica gel and washed with ethyl acetate (400 mL). The solvent was removed under reduced pressure to give a crude that was purified by silica gel column chromatography eluted with ether petroleum–ethyl acetate (90 : 10). Final purification was carried out by semi-preparative HPLC (ether petroleum–ethyl acetate 85 : 15; flow = 3.0 mL min^{-1}) to yield a mixture 9 : 1 of (*E*)-**5** (108.6 mg, 68%) and (*Z*)-**5**.¶

(2*R*,3*R*,4*E*,8*E*)-3-Hydroxy-2,4,8-trimethyldeca-4,8-dienolide ((*E*)-5). Colourless oil; t_R = 28 min; $[\alpha]_D^{20}$ +116° (*c* 0.42 in $CHCl_3$); IR (film) ν_{max}/cm^{-1} 3442 (OH), 2918, 2850, 1731 (CO), 1455, 1374, 1259, 1164, 1024, 904, 794; ^1H NMR (500 MHz, $CDCl_3$) δ_H 1.25 (3H, d, *J* 6.5, 2-Me), 1.61 (3H, s, 4-Me), 1.71 (3H, s, 8-Me), 1.94–2.02 (1H, m, 7b-H), 2.02–2.11 (1H, m, 6b-H), 2.13–2.19 (1H, dt, *J* 11.6, 4.0, 7a-H), 2.41 (1H, dq, *J* 12.2, 4.0, 6a-H), 2.64 (1H, dq, *J* 10.0, 6.5, 2-H), 3.93 (1H, d, *J* 10.0, 3-H), 4.33 (1H, t, *J* 10.6, 10b-H), 4.68 (1H, dd, *J* 10.6, 6.2, 10a-H), 5.02 (1H, dd, *J* 11.7, 4.0, 5-H), 5.50 (1H, m, 9-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 10.2 (q, 4-Me), 13.8 (q, 2-Me), 15.6 (q, 8-Me), 25.7 (t, C6), 38.2 (t, C7), 44.8 (d, C2), 60.9 (t, C10), 82.2 (d, C3), 122.2 (d, C9), 127.9 (d, C5), 136.4 (s, C4), 143.6 (s, C8), 175.0 (s, C1); HRMS (CI^+): calcd for $C_{13}H_{20}O_3$ $[M]^+$ 224.1412, found 224.1397.

Mutasynthesis experiments

General methods. *B. cinerea* was grown on a surface culture in Roux bottles on a Czapek-Dox medium (150 mL per flask) comprising (per L of distilled water) glucose (50.0 g), yeast extract (1.0 g), KH_2PO_4 (5.0 g), $NaNO_3$ (2.0 g), $MgSO_4{\cdot}7H_2O$

¶(*Z*)-**5** could not be purified.

(0.5 g), and $FeSO_4{\cdot}7H_2O$. The pH of the medium was adjusted to 7.0 with aqueous NaOH (4 M). Each Roux bottle was inoculated with 2×10^6 fresh conidia or six uniform discs of 0.9 cm diameter mycelia of four-day old culture on malta agar. A filter-sterilised aqueous solution of the labeled precursor or a solution of (*E*)-**5** in ethanol was fed at a carefully determined optimum time. Roux bottles were incubated at 25 ± 2 °C in daylight under static conditions for the optimum period of time. The culture medium and mycelia were then separated by filtration. The broth was separated with NaCl and extracted with ethyl acetate (3×) and dried over anhydrous Na_2SO_4. The organic extract obtained was evaporated under reduced pressure to dryness.

Feeding of (2*R*,3*R*,4*E*,8*E*)-3-hydroxy-2,4,8-trimethyldeca-4,8-dienolide ((*E*)-5) to *B. cinerea bcbot2Δ* and *bcΔΔd1*. Compound (*E*)-**5** (120 mg), dissolved in EtOH (960 μL), was distributed among 6 Roux bottles containing a 4-day old culture of *B. cinerea bcbot2Δ* or *bcΔΔd1* and grown for a further 6 days. Filtration, ethyl acetate extraction and column chromatography, followed by analytical HPLC purification, gave **11**, **12**, **13** and **14** in the yields shown in Table 1.

(2*R*,3*R*,4*E*,8*S*,9*S*)-8,9-Epoxy-3-hydroxy-2,4,8-trimethyldec-4-enolide (11). Colourless oil; t_R = 27 min, petroleum ether–ethyl acetate (77 : 23), flow = 0.8 mL min^{-1}; $[\alpha]_D^{20}$ +184° (*c* 0.34 in $CHCl_3$); IR (film) ν_{max}/cm^{-1} 3448 (OH), 1734 (CO), 1458, 1165, 1022, 887, 764; ^1H NMR (400 MHz, $CDCl_3$) δ_H 1.08 (3H, dt, *J* 13.4, 4.8, 7b-H), 1.28 (3H, d, *J* 6.6, 2-Me), 1.31 (3H, s, 8-Me), 1.75 (3H, t, *J* 1.6, 4-Me), 2.08–2.16 (2H, m, 6b-H, 7a-H), 2.37–2.48 (1H, m, 6a-H), 2.72 (1H, dq, *J* 10.0, 6.6, 2-H), 3.01 (1H, dd, *J* 10.0, 4.2, 9-H), 3.53 (1H, dd, *J* 10.8, 10.0, 10b-H), 3.99 (1H, dd, *J* 10.0, 1.8, 3-H), 4.86 (1H, dd, *J* 10.8, 4.2, 10a-H), 5.17 (1H, ddd, *J* 12.0, 3.2, 1.6, 5-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 10.3 (q, 4-Me), 13.6 (q, 2-Me), 15.9 (q, 8-Me), 24.3 (t, C6), 37.0 (t, C7), 43.6 (d, C2), 58.6 (d, C9), 59.8 (s, C8), 62.9 (t, C10), 81.6 (d, C3), 126.9 (d, C5), 136.2 (s, C4), 174.4 (s, C1); HRMS (CI^+): calcd for $C_{13}H_{20}O_4$ $[M]^+$ 240.1362, found 240.1353.

(2*R*,3*R*,4*E*,7*R*,8*E*)-3,7-Dihydroxy-2,4,8-trimethyldeca-4,8-dienolide (12). Colourless oil; t_R = 28 min, petroleum ether–ethyl acetate (53 : 47), flow = 0.8 mL min^{-1}; $[\alpha]_D^{20}$ +219° (*c* 0.31 in $CHCl_3$); IR (film) ν_{max}/cm^{-1} 3395 (OH), 2923, 1707 (CO), 1458, 1354, 1258, 1168, 1021, 935, 851, 750; ^1H NMR (400 MHz, $CDCl_3$) δ_H 1.24 (3H, d, *J* 6.6, 2-Me), 1.64 (3H, t, *J* 1.2, 4-Me), 1.75 (3H, d, *J* 1.2, 8-Me), 2.25–2.32 (1H, m, 6b-H), 2.46 (1H, ddd, *J* 13.6, 12.0, 11.2, 6a-H), 2.61 (1H, dq, *J* 10.2, 6.6, 2-H), 3.90 (1H, d, *J* 10.2, 3-H), 4.18 (1H, dd, *J* 11.2, 5.6, 7-H), 4.37 (1H, t, *J* 10.2, 10b-H), 4.70 (1H, dd, *J* 10.2, 6.0, 10a-H), 5.02 (1H, dd, *J* 12.0, 2.4, 5-H), 5.62 (1H, dd, *J* 10.2, 6.0, 9-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 10.1 (q, C8), 10.2 (q, C4), 13.8 (q, C2), 34.5 (t, C6), 44.0 (d, C2), 60.1 (t, C10), 77.2 (d, C7), 81.9 (d, C3), 121.2 (d, C9), 124.7 (d, C5), 137.3 (s, C4), 145.2 (s, C8), 174.8 (s, C1); HRMS (CI^+): calcd for $C_{13}H_{19}O_4$ $[M - H]^+$ 239.1283, found 239.1283.

(2*R*,3*R*,4*E*,7*R*,8*S*,9*S*)-8,9-Epoxy-3,7-dihydroxy-2,4,8-trimethyldec-4-enolide (13). Colourless oil; t_R = 31 min, petroleum ether–ethyl acetate (53 : 47), flow = 0.8 mL min^{-1}; $[\alpha]_D^{20}$ +94.1° (*c* 0.47 in $CHCl_3$); IR (film) ν_{max}/cm^{-1} 3431 (OH), 2922, 2855 (CH), 1716 (CO), 1448, 1373, 1254, 1166, 1019, 848, 741; ^1H NMR (400 MHz, $CDCl_3$) δ_H 1.28 (3H, d, *J* 6.8, 2-Me), 1.34 (3H, s, 8-Me), 1.77 (3H, d, *J* 1.6, 4-Me), 2.28–2.35 (1H, m, 6b-H), 2.50 (1H, dt, *J* 13.6, 11.8, 6a-H), 2.69 (1H, dq, *J* 9.8, 6.8, 2-H), 3.09 (1H, dd, *J* 10.0, 4.0, 9-H), 3.22 (1H, dd, *J* 11.8, 5.2, 7-H), 3.56 (1H, dd, *J* 10.8, 10.0, 10b-H), 3.97 (1H, d, *J* 9.8, 3-H), 4.89 (1H, dd, *J* 10.8, 4.0, 10a-H), 5.12 (1H, dd, *J* 11.8, 3.2, 5-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 10.2 (q, 8-Me), 10.5 (q, 4-Me), 13.6 (q, 2-Me), 32.5 (t, C6), 43.4 (d, C2), 56.6 (d, C9), 62.2 (t, C10), 62.6 (s, C8), 76.9 (d, C7), 81.4 (d, C3), 122.5 (d, C5), 137.6 (s, C4), 174.4 (s, C1); HRMS (CI^+): calcd for $C_{13}H_{20}O_5$ $[M]^+$ 256.1311, found 256.1310.

(2*R*,3*R*,4*E*,6*R*,8*S*,9*S*)-8,9-Epoxy-3,6-dihydroxy-2,4,8-trimethyldec-4-enolide (14). Colourless oil; t_R = 38 min, petroleum ether–ethyl acetate (30 : 70), flow = 0.8 mL min^{-1}; $[\alpha]_D^{20}$ +46.3° (*c* 0.11 in $CHCl_3$); IR (film) ν_{max}/cm^{-1} 3422 (OH), 2921, 2856 (CH), 1719 (CO), 1458, 1387, 1259, 1172, 1028, 932, 824; ^1H NMR (600 MHz, $CDCl_3$) δ_H 1.12 (1H, t, *J* 11.2, 7b-H), 1.30 (3H, d, *J* 6.6, 2-Me), 1.32 (3H, s, 8-Me), 1.83 (3H, d, *J* 1.2, 4-Me), 2.48 (1H, dd, *J* 11.2, 4.8, 7a-H), 2.72 (1H, dq, *J* 10.2, 6.6, 2-H), 3.05 (1H, dd, *J* 10.2, 4.2, 9-H), 3.49 (1H, dd, *J* 10.8, 10.2, 10b-H), 4.00 (1H, dd, *J* 10.2, 1.2, 3-H), 4.64 (1H, dt, *J* 11.2, 4.8, 6-H), 4.90 (1H, dd, *J* 10.8, 4.2, 10a-H), 5.20 (1H, d, *J* 11.2, 5-H); ^{13}C NMR (150 MHz, $CDCl_3$) δ_C 10.7 (q, 4-Me), 13.5 (q, 2-Me), 17.1 (q, 8-Me), 43.7 (t, C7), 45.6 (d, C2), 58.0 (s, C8), 58.6 (d, C9), 62.8 (t, C10), 65.9 (d, C6), 80.9 (d, C3), 129.4 (d, C5), 139.0 (s, C4), 174.1 (s, C1); HRMS (CI^+): calcd for $C_{13}H_{21}O_5$ $[M + H]^+$ 257.1389, found 257.1387.

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